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The Application of the Haemagglutination Test to a Study of the Immunity to Malaria in Protected and Unprotected Population Groups in Australian New Guinea*

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The formolized tanned sheep erythrocyte haemagglutination test has been applied to an immuno-malariometric study in Australian New Guinea to determine whether the haemagglutination titre reflects a subject's immune state and to measure the effect of malaria control operations on a population's immunity. Two population groups were studied—one (unprotected) living in holoendemic malaria conditions, the other (protected) living in an area subject to malaria control measures for four years.

An increase in both serological positivity rates and geometric mean titres among the unprotected group with increasing age suggests that the test does serve to assess the state of immunity; the corresponding rates were much lower in the protected population, particularly among the children.

The authors foresee the possible use of the haemagglutination test as a supplement to other procedures in assessing the progress of a malaria campaign. They, note, however, that more immuno-malariometric studies on populations subject to different degrees of malaria endemicity will need to be carried out before the relationship between the immune state and serological results can be clearly established.

Our knowledge of the manner in which man acquires his immune defence against malaria has been largely based on the indirect evidence of clinical and epidemiological observations. Indeed, it is fortunate that malaria is a type of infection in which the manifestations of immunity can be so readily studied; parasitaemia rates and densities, mortality and morbidity in a population, as well as the spleen rates, all provide important insights into the dynamics of human malarial immunity.

There is, however, a notable absence of supportive serological data, mainly due to the lack of a suitable specific technique to detect and measure humoral antibody elaborated in human malaria. The complement-fixation test has not found widespread application, one reason being the relatively large amount of antigen required. The fluorescent antibody technique (Tobie & Coatney, 1961; Ingram et al., 1961; Tobie et al., 1962; Voller & Bray, 1962) shows more promise in this direction, although possible objections to the method are the high cost of equipment and the difficulty in subjectively assessing antibody titre. However, Bray (1962) investigated a small group of Liberians and found that the immunofluorescence titres agreed with the known relationship of age to immunity.

A tanned sheep erythrocyte haemagglutination test for the measurement of antibody in malaria infections has been described by Desowitz & Stein (1962) and Stein & Desowitz (1964). The test appears to be highly sensitive and has the further advantage of requiring only minute amounts of antibody-antigen reactants. The technique having been stabilized to yield satisfactorily reproducible results, it was decided to supplement the laboratory investigations with field studies. The purpose was, primarily, to determine whether, in a population living under conditions of hyper- or holoendemic

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malaria, the immune response as exemplified by haemagglutination titres could be related to factors known to influence or reflect the degree of immunity —e.g., age, gamma-globulin levels, parasite rates, etc. A secondary aim was to carry out a similar study in an area where malaria had been eradicated or was at some stage in an eradication programme. The purpose of this latter study was not only to determine what happens to humoral antibody when control measures are instituted but also to investigate the possibility of using the haemagglutination test as a supplement to microscopic blood examinations in assessing the progress of a malaria eradication campaign.

The area chosen for the study was near Maprik in the Sepik District of Australian New Guinea. A considerable amount of epidemiological information on this area was already available from the investigations of Peters (1960) and Peters & Standfast (1957, 1960). Adjacent to a population living under conditions of holoendemic malaria was another group in a former pilot project and now under protection from malaria.

MATERIALS AND METHODS

Population studied

Unprotected group. The unprotected group consisted of some 1000 people living in hamlets surrounding Salata village. There is perennial malaria transmission, of varying intensity, maintained by *Anopheles punctulatus*, *A. farauti* and *A. koliensis*. The Salata people appeared to be relatively well nourished. The disease pattern revealed the presence of hookworms, tuberculosis, yaws, respiratory disorders, tropical ulcers, tinea imbricata and filariasis, as well as malaria. The pregnancy rate is apparently low and A. D. Parkinson [1] calculated the first year survival rate to be 68.9% per 1000 live-births.

Prior to our survey of August 1963, there had been no antimalarial operations, such as DDT indoor spraying or mass drug administration. The basic health services are provided by government aid posts, mission stations and the government hospital at Dreikikir. For those who seek medical aid for " fever ", chloroquine, quinine, or both, is routinely given and undoubtedly an undetermined number of the population have been so treated. It will be seen from the parasite rates in Table 1 below that the effect of such sporadic drug administration on the prevalence of malaria must be negligible.

Protected group. This population group is situated within the Maprik area, approximately 15 miles (24 km) from the Salata unprotected group. Control measures, which commenced in 1959, consist of twice-yearly spraying with DDT and mass drug administration. The drug used is a combination of chloroquine 125 mg and pyrimethamine 25 mg in tablet form. Non-febrile adults receive two tablets and febrile adults double this dosage. Infants receive a quarter of a tablet, toddlers half a tablet, and children up to 15 years one tablet. The last spraying and mass drug administration were undertaken in December 1963.

Survey methods

Unprotected group. A preliminary parasitological and clinical survey of the unprotected Salata group was made in June 1963. The names of the inhabitants of the hamlets were recorded on individual punch cards. Age, sex, village, and mother's and father's names were also recorded. Examinations for splenic enlargement, using Hackett's (1944) method of assessment, and for liver enlargement in centimetres were also carried out at this time. A thick blood film and a thin film on one slide were made from each individual. The slides were taken to the Central Malaria Laboratory, Moresby-Konedobu, and stained with Giemsa stain. One hundred fields were examined microscopically and the parasitological findings entered on the individual punch cards.

In August 1963, the second survey of the Salata group was undertaken and it was at this time that serum for serological testing was obtained. Blood was collected by bleeding into either sterile MacCartney bottles or withdrawing into disposable plastic syringes. The latter method was found to be the more efficient, with rapid and good clot formation in the disposable syringes. The blood samples were allowed to clot in the syringe for approximately eight hours, after which time the syringe was opened with a cutter and the serum removed with a sterile Pasteur pipette or simply expressed directly into an individually coded sterile test-tube. Bending the needle after blood withdrawal appeared to prevent contamination. The test-tubes containing the sera were stored in a portable butane-operated refrigerator for not more than two days, after which they were sent to the Malaria Laboratory in Maprik,

[1] Unpublished report to the Department of Public Health, Territories of Papua and New Guinea.

where they were stored at −20°C. The sera were finally taken in dry-ice to Singapore, where the serological studies were performed. Blood films were also taken and processed in the same way as those of the June survey, the only difference being that 200 instead of 100 fields were examined.

Protected group. This survey was undertaken after the main investigation of the unprotected group was completed. It was of a preliminary nature and the number of sera obtained was less than that for the unprotected population. Serum and blood films were taken in August 1963 but spleen and liver examinations were not carried out until February 1964.

Serological studies

Haemagglutination test. A detailed account of the haemagglutination test employing formolized, tanned sheep erythrocytes has been given by Stein & Desowitz (1964), and it was this technique that was used in this present study.

Each serum sample was tested with two simian malaria antigens, *Plasmodium cynomolgi* and *P. coatneyi*. The results of current investigations have indicated that *P. cynomolgi* is antigenically related to *P. vivax* and *P. coatneyi* to *P. falciparum*. There is some cross-reaction, particularly with high-titre sera. For example, *P. cynomolgi*-sensitized cells may give a titre of 1/12 800 with serum from a patient infected with *P. vivax* but only 1/800 with *P. coatneyi* antigen. It will be seen that a relatively large proportion of the unprotected popoulation was infected with *P. malariae*. However, it is, as yet, not known if there are any cross-reactions of antibody to this parasite with the antigens of the two simian plasmodia used in these studies.

Before use, the antigen was stored at −70°C, a temperature which seems to preserve its potency for at least six months. The serum samples were stored at −20°C. After testing normal sera obtained from a Swiss blood bank and from British soldiers, it was found that highly chylous, turbid sera gave false-positive reactions. Accordingly, the few turbid sera of this survey were discarded. In the previous account of the test (Stein & Desowitz, 1964) it was noted that a certain proportion of the control sera gave positive, generally low-titre, reactions. These control sera were obtained from the Singapore blood bank, and it was suggested that since malaria is present in adjacent areas the positive controls might have come from individuals who have had some previous contact with the infection. Since that time sera have been obtained from persons known to have had no contact with malaria. Nine serum samples were obtained from a Swiss blood bank and all but the one chylous, possibly contaminated, serum gave negative reactions. Similarly, of 11 sera from British soldiers who had come directly to Singapore from Britain, 10 were negative and one gave a titre of 1/100.

Serum protein analysis. Serum proteins were determined by Antweiler micro-Tiselius moving-boundary electrophoresis. Quantitative estimates of total protein and of individual electrophoretic fractions were made by interferometric analysis. It was not possible to analyse all the sera collected and a randomly selected sample was tested.

RESULTS

Parasitology

Unprotected group. The parasitological data obtained from the June 1963 and August 1963 surveys are summarized in Table 1. For comparative purposes the unprotected population has been divided into six age-groups: 1-2 years, 3-4 years, 5-6 years, 7-9 years, 10-15 years and adults (over 15 years). The figures from both surveys reveal a high parasite rate in children with a gradual decrease in the older age-groups, the lowest parasite rates being present in the adult group. The differences between the 1-2-year group and the adults were 70.58% and 12.08% (June) and 84.00% and 39.80% (August) respectively. Not only was the rate higher for all age-groups in the August survey but there was also a shift in the parasite rates. There are three main possible explanations for these variations. First, 200 microscopic fields per thick film were examined in August, whereas only 100 fields were examined in June. It is probable that more extensive examination would yield higher positivity rates. Secondly, although there is perennial transmission in this area of New Guinea, its intensity is determined by climatic factors influencing vector density. This was evidenced by the entomological search [1] in the area preceding the June survey. At this time the dry weather conditions had reduced the vector population considerably. Increased rainfall in July and August ensured a more favourable environment for the local anopheline vectors. Thirdly, the shift in

[1] J. H. Bryan & W. Jeffery—personal communication, 1963.

TABLE 1

PARASITE RATES IN JUNE 1963 AND AUGUST 1963 SURVEYS AMONG THE UNPROTECTED POPULATION

	Age-group (years)												Total	
	1-2		3-4		5-6		7-9		10-15		Adults (over 15)			
	June	August	June	August	June	August	June	August	June	August	June	August	June	August
No. examined	17	25	35	40	27	41	27	39	39	44	149	201	294	390
Positive (all forms)	12 (70.58 %)	21 (84.00 %)	27 (77.14 %)	33 (82.50 %)	15 (55.55 %)	34 (82.92 %)	16 (59.26 %)	26 (66.66 %)	16 (41.02 %)	25 (56.81) %	18 (12.08 %)	80 (39.80 %)	104 (35.38 %)	219 (56.16 %)
Positive *P. falciparum*	10 (58.82 %)	6 (24.00 %)	21 (60.00 %)	7 (17.50 %)	13 (48.15 %)	8 (19.51 %)	12 (44.44 %)	5 (12.82 %)	12 (30.77 %)	6 (13.63 %)	15 (10.06 %)	21 (10.44 %)	83 (28.23 %)	53 (13.58 %)
Positive *P. vivax*	1 (5.88 %)	5 (20.00 %)	2 (5.71 %)	6 (15.00 %)	0	6 (14.63 %)	2 (7.40 %)	1 (2.56 %)	1 (2.56 %)	6 (13.63 %)	3 (2.01 %)	14 (6.96 %)	9 (3.06 %)	38 (9.74 %)
Positive *P. malariae*	1 (5.88 %)	2 (8.00 %)	4 (11.42 %)	12 (30.00 %)	1 (3.70 %)	7 (17.07 %)	1 (3.70 %)	13 (33.33 %)	3 (7.69 %)	8 (18.18 %)	0	41 (20.40 %)	10 (3.40 %)	83 (21.28 %)
Positive *P. falciparum* + *P. vivax*	0	1 (4.00 %)	0	1 (2.50 %)	1 (3.70 %)	0	0	1 (2.56 %)	0	2 (4.54 %)	0	1 (0.50 %)	1 (0.34 %)	6 (1.54 %)
Positive *P. falciparum* + *P. malariae*	0	3 (12.00 %)	0	6 (15.00 %)	0	11 (26.83 %)	1 (3.70 %)	3 (7.69 %)	0	1 (2.27 %)	0	3 (1.49 %)	1 (0.34 %)	27 (6.92 %)
Positive *P. vivax* + *P. malariae*	0	3 (12.00 %)	0	1 (2.50 %)	0	1 (2.44 %)	0	3 (7.69 %)	0	2 (4.54 %)	0	0	0	10 (2.56 %)
Positive *P. vivax* + *P. falciparum* + *P. malariae*	0	1 (4.00 %)	0	0	0	1 (2.44 %)	0	0	0	0	0	0	0	2 (0.51 %)

parasite rates is indicative of the uncertainties accompanying many malaria prevalence studies. The influence of intermittent treatment provided by the basic health services must also be considered. Some differences in the results of the two surveys cannot be readily accounted for. There is a distinct decrease in the number of *P. falciparum* infections in August as compared to June. However, the number of *P. malariae* infections, both as single and mixed infections, rose in August. To a lesser degree there was also a rise in *P. vivax* infections during this period. Ratios for over-all parasite rates of *P. falciparum*, *P. vivax* and *P. malariae* respectively were 80.2 : 9.4 : 10.4 in June and 33.3 : 22.2 : 44.4 in August. There was a considerable number of individuals whose blood slide was positive for one species in June and another two months later. Furthermore, some individuals found to be negative at one survey were found to be positive for one or more species at the other survey.

Protected group. As noted previously the serological survey of the protected group was less comprehensive than that of the unprotected population. The parasitological data, summarized in Table 2, were obtained from a routine survey carried out in June 1963. It will be seen that the combined mass drug administration and spraying programme of the previous four years had resulted in a notable reduction of the parasite rate in all age-groups. Before control measures were begun the parasite rates were generally similar to those for the unprotected group described above (Peters & Standfast, 1957). Although parasite rates were low, it is interesting to note that *P. malariae* infections were completely absent and *P. falciparum* and *P. vivax* occurred in a proportion of 13: 8.

Spleen and liver examinations

Results of the spleen and liver examinations of the unprotected and protected populations are shown in Table 3. The spleen rate of the unprotected population declined with the advance of age but the average enlarged spleen size was approximately the same for all age-groups; the spleen rate was extremely high (100%-80%) up to the age of 10 years; thereafter there was a marked reduction in the rate (57% in the 10-15 age-group and 37% in adults). Liver enlargement rates were, for all age-groups, lower than the spleen rate, but here too there was a decrease with advancing age.

Spleen and liver examinations of the protected population were carried out by Dr J. Dewdney, Public Health Department, Maprik, in February 1964. Dr Dewdney divided the population into two age-groups only, 2-9 years and over 9 years; nevertheless, comparison of the upper and lower parts of Table 3 shows the striking decrease in spleen and liver rates of the protected population in con-

TABLE 2

PARASITE RATES IN JUNE 1963 AMONG THE PROTECTED POPULATION

	Age-group (years)							
	<1	1-2	3-4	5-6	7-9	10-15	Adults (over 15)	Total
No. examined	26	99	139	49	75	94	193	675
Positive (all forms)	0	2 (2.02 %)	2 (1.44 %)	5 (10.20 %)	4 (5.33 %)	4 (4.25 %)	4 (2.07 %)	21
Positive *P. falciparum*	0	1 (1.01 %)	1 (0.62 %)	3 (6.12 %)	4 (5.33 %)	1 (1.06 %)	3 (1.56 %)	13
Positive *P. vivax*	0	1 (1.01 %)	1 (0.72 %)	2 (4.08 %)	0	3 (3.19 %)	1 (0.51 %)	8
Positive *P. malariae*	0	0	0	0	0	0	0	0
Mixed infections	0	0	0	0	0	0	0	0

TABLE 3
SPLEEN AND LIVER RATES OF THE UNPROTECTED AND PROTECTED POPULATIONS

Age-group (years)	Spleen			Liver		
	No. examined	Spleen rate (%)	Average enlarged spleen	No. examined	Liver rate (%)	Average enlarged liver (cm)
Unprotected population						
1-2	17	100	2.42	17	58.8	1.60
3-4	38	94.7	2.05	39	41.0	1.62
5-6	34	82.3	2.14	33	36.4	2.00
7-9	36	80.5	1.96	34	44.1	1.74
10-15	42	57.1	2.00	42	30.9	2.00
Adults	154	37.0	2.21	163	25.7	2.92
Protected population						
2-9	143	14.0	1.2	143	2.8	2.0
Over 9	458	7.7	1.3	548	7.2	3.3

trast to the unprotected population. This decline in liver and spleen rates was apparent for both children and adults.

Haemagglutination titres

Unprotected group. In view of the well-established relationship of age and immunity to malaria, analysis of the haemagglutination titres was carried out on this basis. Statistical analysis was performed by the World Health Organization, Geneva. The upper portion of Table 4 shows the percentage distribution of tires for various age-groups of the unprotected population. A certain percentage of each group was negative to one or both antigens. Statistical analysis of the positivity rate in relation to age showed no significant differences between the five age-groups of children. The values of χ^2 are: *P. cynomolgi*, $\chi^2=0.68$ (not significant); *P. coatneyi*, $\chi^2=2.16$ (not significant). However, when the positivity rates of adults are compared with the entire group of children then there is a significant difference. The χ^2 values here are: *P. cynomolgi*, $\chi^2=10.33$, $0.001<P<0.01$; *P. coatneyi*, $\chi^2=9.35$, $0.001<P<0.01$. Among the various age-groups the positivity rate ranged from 70.8% to 92.2%. While the majority of the negative serological reactants was also parasitologically negative, there were a few cases where the negative reaction was accompanied by a positive blood film.

The geometric mean titre for each age-group is shown in the upper portion of Table 5. It will be seen that there was, for both antigens, a general increase in mean titre with the progression of age. The most notable increase of mean titre appeared to be between the ages of 1-2 and 3-4. Statistical analysis shows the logarithmic titres to be of normal distribution and the differences among the mean titres were tested by the analysis of variance. While there is a trend of progressive increase of titre with age, statistical analysis indicates no significant differences among the five age-groups of children with *P. cynomolgi* antigen but there is a significant difference with *P. coatneyi* antigen ($0.01<P<0.05$). Because of the composition of the population sampled, the number in the children's age-groups was relatively small, and additional studies will have to be performed to give statistical confirmation to the observed trend of higher titres in the older age-groups. Statistical evidence of age differences was obtained when the geometric mean titres of all children (1-10 years) and the adult group were compared. Here, with *P. cynomolgi* there was a significant difference ($0.001<P<0.01$).

Protected population. The difference in serological positivity rates between the 1-2-year-old and 3-4-year-old groups is not significant. However, the difference in positivity rate between adults and all children is statistically significant ($P<0.001$ with *P. cynomolgi*, and $0.001<P<0.01$ with *P. coatneyi*).

The lower portion of Table 4 shows the percentage distribution of titres for the protected population,

TABLE 4. PERCENTAGE DISTRIBUTION OF HAEMAGGLUTINATION TITRES FOR VARIOUS AGE-GROUPS OF THE UNPROTECTED AND PROTECTED POPULATIONS

Age-group (years)	No. examined	Titre [a] (reciprocal)																							
		Negative		100		200		400		800		1 600		3 200		6 400		12 800		25 600		51 200	
		Cy	Co	Cy	Co	Cy	Co	Cy	Co	Cy	Co	Cy	Co	Cy	Co	Cy	Co	Cy	Co	Cy	Co	Cy	Co
Unprotected population																									
1-2	28	21.42	17.86	0	0	7.17	10.71	7.14	7.14	21.42	7.14	14.28	28.52	14.28	14.28	7.14	3.57	3.57	7.14	3.57	3.57	0	0
3-4	48	20.83	29.17	0	0	4.16	2.08	4.16	4.16	10.41	10.41	14.58	6.25	12.50	18.75	10.41	6.25	10.41	16.66	6.25	4.16	6.25	2.08
5-6	50	18.00	18.00	0	2.00	0	6.00	6.00	8.00	12.00	4.00	20.00	18.00	8.00	12.00	16.00	10.00	12.00	14.00	6.00	6.00	2.00	2.00
7-9	44	15.90	22.73	0	0	2.27	2.27	4.54	2.27	9.09	11.36	15.90	9.09	13.64	13.64	9.09	18.18	6.81	6.81	13.64	11.36	9.09	2.27
10-15	54	16.66	22.22	0	0	1.85	3.70	3.70	0	9.26	5.55	14.81	7.40	9.26	22.22	20.37	16.67	11.11	12.96	7.41	3.70	5.55	5.55
Adults (over 15)	232	7.76	11.20	0.43	1.29	2.15	1.29	4.30	4.30	8.18	7.32	12.93	15.08	12.93	15.08	16.37	18.53	16.81	14.22	6.70	6.70	10.77	5.17
Protected population																									
1-2	26	73.08	73.08	0	0	11.54	0	7.69	7.69	3.84	7.69	3.84	7.69	0	3.84	0	0	0	0	0	0	0	0
3-4	18	72.23	55.55	0	0	5.55	0	5.55	0	11.11	11.11	0	27.77	5.55	5.55	0	0	0	0	0	0	0	0
Adults (over 15)	27	22.22	25.92	0	0	0	0	3.70	3.70	11.11	18.51	14.81	3.70	25.92	29.62	11.11	7.40	3.70	3.70	7.40	0	0	7.40

[a] Cy = *P. cynomolgi* antigen; Co = *P. coatneyi* antigen.

TABLE 5

GEOMETRIC MEAN TITRES OF POSITIVE SERA FOR VARIOUS AGE-GROUPS OF THE UNPROTECTED AND PROTECTED POPULATIONS

Age-group (years)	Geometric mean titre	
	P. cynomolgi antigen	*P. coatneyi* antigen
Unprotected population		
1-2	1 550	1 650
3-4	3 510	3 620
5-6	3 310	2 700
7-9	4 570	4 260
10-15	4 200	4 720
All children	3 460	3 340
Adults over 15	5 060	4 170
Protected population		
1-2	400	975
3-4	696	1 470
Subtotal, children 1-4	504	1 210
Adults	3 000	2 990

and the mean titres of the positive cases are given in the lower portion of Table 5. As in the unprotected group the trend, even though the sampling is limited, is towards higher titres as age increases. The difference between the two groups of children is not statistically significant but the difference in mean geometric titre between the children and adults is $P<0.001$ with *P. cynomolgi* and $0.01<P<0.05$ with *P. coatneyi*.

Comparison of unprotected and protected groups. It will be seen from comparison of the two parts of Table 4 that the serological positivity rate in all instances is lower in the protected group than in the unprotected. The difference is most marked in the 1-4-year age-groups, where the positivity of the unprotected population is some 40%-50% higher than in the protected population. The order of difference is approximately the same with both antigens. The disparity of positivity rates between the adult groups is about 15% less than that for the children of the two populations. There is a statistically significant difference between the protected and unprotected populations for both children and adults—$P<0.001$ for children and $0.01<P<0.05$ for adults.

Not only was there a much higher percentage of negative sera from the protected group but also the mean titre of the positive cases was, for each age-group and with both antigens, lower than the mean titre of the comparable age-group in the unprotected population. Again the difference was most marked between children, this being particularly true for the mean titres obtained with *P. cynomolgi* antigen. While there are considerable differences between the mean *P. coatneyi* titres of the children's groups, these are not statistically significant. However, the differences in mean titre between these same age-groups are, with *P. cynomolgi* antigen, statistically significant ($P<0.001$). Differences between the adults of the two populations were not statistically significant.

Serum proteins

Table 6 compares the average serum protein values of the various age-groups of the protected and unprotected populations. In the unprotected population the γ-globulin level was relatively high in all age-groups, ranging from 2.03 g/100 ml in the 1-2-year-old group to 2.52 g/100 ml in the adults. In three age-groups tested in the protected population (1-2 years, 3-4 years and adults), the average γ-globulin level was, in all cases, considerably lower than that of comparable unprotected populations, these being 1.33 g/100 ml, 1.40 g/100 ml and 1.80 g/100 ml respectively. There does not appear to be any marked difference in the β-globulin between the two populations. The average α-globulin and albumin levels were slightly higher in all three age-groups of the protected population than those in the comparable age-groups in the unprotected population.

DISCUSSION

While serological techniques can be applied to detect the presence of antibody they do not necessarily provide information regarding the subject's actual state of immunity. In view of this, a major object of the present study has been to determine whether the haemagglutination test can serve as an indicator of the process by which a population living under conditions of holoendemic malaria develops an immunological competency.

It is well known that there is an association between age and immunity to malaria. The gradual reduction in parasite and spleen rates in the older groups of the unprotected Salata community would indicate that this factor is operative in this population also. Age, therefore, has been used as a convenient criterion in interpreting the results of the haemagglutination technique. That the haemagglutination titre does bear a quantitative relationship to immunity is indicated by the progressive increase of geometric mean titre from the youngest group (1-2 years) to the adults. It is possible that the

TABLE 6

ANALYSIS OF SERUM PROTEINS IN EACH AGE-GROUP OF THE UNPROTECTED AND PROTECTED POPULATIONS

Age-group (years)	Population	No.	Average serum proteins (g/100 ml)				
			γ-globulin	β-globulin	α-globulin	Albumin	Total protein
1-2	Unprotected	16	2.03	1.09	0.93	3.35	7.37
	Protected	8	1.22	1.04	1.18	3.90	7.45
3-4	Unprotected	30	2.37	0.94	0.90	3.25	7.44
	Protected	14	1.40	0.86	1.16	3.48	6.90
5-6	Unprotected	26	2.44	1.02	0.75	3.21	7.42
7-9	Unprotected	24	2.25	1.11	0.85	3.12	7.33
10-15	Unprotected	29	2.22	1.14	0.75	3.25	7.37
Adults (over 15)	Unprotected	47	2.52	1.37	0.58	2.98	7.58
	Protected	23	1.84	1.18	0.85	3.46	7.36

development of humoral antibody, as measured by the haemagglutination test, does not proceed at an even rate. The lowest titres were found in the 1-2-year-old group. By 3-4 years, the average of 1500-1600 (the reciprocal of titre is used in all cases) of the 1-2-year-olds is increased to 3500-3600. This average titre is maintained until about 6 years of age, after which it increases again to 4100-4700. With *P. coatneyi* antigen, presumptively specific for antibody to *P. falciparum*, this average level is present from 7-9 years into adulthood. With *P. cynomolgi* antigen, as a measure of antibody to *P. vivax*, an average titre of 4200-4500 persists from 7-9 years until 10-15 years, after which time it rises again to 5060 in the adult group. In the 7-9-year-old group onwards, the period of second rise in average titre, there is a considerable reduction in parasite rates. Determination of parasite densities would probably give additional information on the effect of various antibody titres, particularly the titres found for the 3-6-year-old group, in which the parasite rates remain similar to the 1-2-year-old group.

It is possible that the average antibody titre alone is not solely responsible for affecting the parasitaemia, but the maximum limit of antibody production which the individual is capable of producing may also be operative. Desowitz (1959) showed that the immunological control of bovine trypanosomiasis appeared to be dependent upon the dual factors of over-all average titre and maximum titres elicited during the course of infection. In the present investigation, if the three highest titres—12 800, 25 600 and 51 200—are taken collectively as representing the possible limit of antibody production during the course of infection in any single age-group, it will be seen that 7%-10% of the 1-2-year-old group had high-titre sera, while 20%-22% of the 3-6-year-olds, 22%-29% of the 7-15-year-olds and 30%-34% of the adults exhibited this level of antibody. The sera were collected within a relatively short space of time and serological data from these sera, for any specific age-group, might conceivably represent the serological pattern for a single individual over an extended period of time.

The haemagglutination titres show, within any given age-group, a normal distribution, although there are wide variations between maximum and minimum titres. Two explanations may be offered for this phenomenon. First, that in individuals living under conditions of malaria transmission described in this paper, the titre at any given age remains relatively constant. The range of titres found in an age-group would represent, immunologically, " the best that they could do ". The alternative hypothesis is that antibody titre does not remain constant but rather fluctuates within given limits for an age-group during the course of infection. There is evidence that this second explanation is the more likely. Desowitz (1959) has shown that in the early stage of bovine trypanosomiasis, a period characterized by a series of intense parasitic recrudescences, the antibody level, as measured by the respiratory test, fluctuates markedly and may fall to zero immediately before or after a crisis. After chronicity is established, these pronounced fluctuations are no longer apparent. Collins et al. (1964) have shown a similar phenomenon to occur in the primary attack of *P. falciparum* in non-immune volunteers following non-curative chemotherapy. Stein (personal communication) in Singapore has observed that in simian malaria, the haemagglutination titre varies from day to day and may become negative immediately after crisis induced by chemotherapy. Undoubtedly the malaria parasite is in dynamic relationship to the immunological reaction of the host. Subtle changes in the immunological constitution of the host may have a profound effect upon the parasite and the nature of the infection it produces. These immunological changes, particularly those associated with humoral antibody level, may not easily be analysed by statistical treatment and it will undoubtedly require much further experimentation in the laboratory and the field to confirm the pattern of the immune response, as evidenced by the haemagglutination test, that is presented here.

It is obvious from the parasite rates that each individual of the unprotected population has at some time had malaria and is constantly exposed to reinfection. Despite this, a certain proportion of sera in each age-group gave a negative haemagglutination reaction at the initial dilution of 1/100. The highest percentage of negative reactors, 18%-20%, was found in the 1-4-year age-group. After this age there was a gradual decrease in negative reactions until of the adult sera only 8%-11% failed to give a positive haemagglutination reaction. The explanation for this is again postulated on the basis of antibody fluctuation during the course of infection. In bovine trypanosomiasis and in malaria, as far as it is now known, these variations in titre seem to be most marked in the infections of non- or semi-immunes, i.e., where there are intense cycles of parasitic crises and recrudescences. With crisis,

induced by either antibody or chemotherapeutic agents, the humoral antibody seems to be greatly reduced or even to disappear. This may be due to rapid antibody decay or absorption by the released antigen. As has been noted previously, these fluctuations in antibody level are not apparent in chronic infections. Thus in the younger age-groups, in which the infection is of a more intense character, it might be expected that a relatively large proportion would be at a stage in the cycle where antibody might be undetectable, while in the adult group, in whom the infection is generally chronic, a smaller proportion would give a negative serological reaction.

Although the sample of the protected population is limited, the immunological differences between it and the unprotected people are striking. This is particularly true of the children who have grown up under the umbrella of chemotherapeutic and mosquito control measures. For all age-groups the dramatic reduction in the parasite rate is clearly evident. Associated with the decrease in the parasite rate is a concomitant decline in both serological positivity and geometric mean titre, as is evident in the children from 1-4 years, the only age-group of children studied. For the protected children only 27% were positive with *P. cynomolgi* antigen, compared with 79% of unprotected children of comparable age. Similarly the average titre of the positive children was only a quarter of that for the unprotected children. The difference is of a slightly lower order with *P. coatneyi*, which might indicate that under the control measures employed the children are somewhat more at risk to *P. falciparum* infections than to *P. vivax*. While the positivity rates and mean titres of the adult protected population are lower than those of the unprotected population the differences are small in comparison to those between the children. It is possible that the adults, having been continuously exposed to infection until five years ago, maintain a high antibody level, perhaps from a subcurative effect of the chemoprophylactic regimen given only twice a year. It is also probable that the adults, who wander further from the village for hunting and food-gathering, would be more liable to infection than the children, who tend to remain within the confines of the DDT-sprayed village. Other immunological changes in the protected population are reflected in the lowered spleen rates and γ-globulin levels. Undoubtedly the twice-yearly programme of mass drug administration and DDT spraying has produced a notable effect on the immunological pattern of the protected group, particularly children. It must be kept in mind, however, that this is the first application of the haemagglutination test to a field study. Moreover, there has been very little in the way of similar studies using other serological techniques for malaria that may be used for comparison. The factors influencing the immune response in " natural" populations are undoubtedly vastly more complex than those of experimentally induced malarias in man, monkeys or rodents. It will require many future investigations into the natural history of malaria immunity under many different conditions of epidemicity before a clear-cut picture emerges of the relationship between the state of immunity and the results yielded by the serological methods at our disposal.

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RÉSUMÉ

Un des auteurs a décrit précédemment, en collaboration avec Stein, une épreuve d'hémagglutination des hématies de mouton traitées par le formol et l'acide tannique, pour le dosage des anticorps antipaludéens. Cette épreuve a été utilisée au cours d'une enquête immuno-paludométrique (comportant en outre la détermination des indices splénique, hépatique et plasmodique, ainsi que l'étude des protéines sériques), sur deux groupes de population de

Nouvelle-Guinée. Le premier, exposé au paludisme holoendémique, n'avait bénéficié d'aucune mesure de protection; dans le secteur occupé par le second groupe, un traitement médicamenteux de masse et des pulvérisations de DDT étaient effectuées deux fois par an, depuis cinq ans.

Les résultats montrent que l'épreuve d'hémagglutination donne la mesure de l'immunité et permet, indirectement, d'évaluer l'efficacité de la lutte antipaludique. Dans le groupe non protégé, 70,8%-92,2% des réactions étaient positives, avec des pourcentages plus élevés chez les adultes. Le titre moyen augmentait avec l'âge, le plus faible (1: 500) étant enregistré dans le groupe d'âge de 1 à 2 ans, le plus élevé dépassant 1 : 5000 chez les adultes. Dans l'autre groupe, environ 70% des enfants de 1 à 4 ans avaient une réaction négative (17%-29% chez les enfants non protégés du même âge) et la valeur du titre moyen des sérums positifs variait de la moitié au quart de celle observée dans le groupe correspondant n'ayant bénéficié d'aucune protection. Chez les adultes des deux groupes en revanche, la différence entre les valeurs du titre moyen était moins marquée. Les taux de gamma-globulines et les divers indices dans le groupe protégé étaient inférieurs à ceux de l'autre groupe.

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